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2501-Pos Board B271

Effects of KL4-Type Peptides on the Surface Activity and Stability of Pulmonary Surfactant Films as Evaluated in the Captive Bubble Surfactometer

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Although SP-B is the most critical protein in lung surfactant, recombinant or synthetic forms of SP-B as a basis for the development of therapeutic surfactants are still not available. An alternative is the design and production of peptides mimicking the structure and general properties of essential motifs in SP-B.

In the present study the surface activity of different KL4-derived peptides, as sequence variations of the original peptide designed to replicate a general amphipathic motif of SP-B [1], has been assessed in the captive bubble surfactometer. The peptides were reconstituted in a surfactant lipid matrix: DPPC/POPC/POPG (50:25:15, w/w/w). This mixture was selected because it offers a fluid environment where the interfacial stability of surfactant films has to be provided primarily by the SP-B mimetic and not by the lipid moiety.

Presence of just 1% (w/w) peptide KL4 (KLLLLKLLLLKLLLLKLLLLK) provided to the lipid mixture similar ability to adsorb at the interface than the presence of 1% native SP-B purified from porcine lungs. Films made of DPPC/POC/POPG/KL4 showed also similar ability to reach very low surface tension with limited compression than exhibited by films containing native SP-B, both under quasi-static and dynamic compression-expansion cycling. A KL4 peptide with amidated end showed similar surface activity, as well as a KL4 version including a PQ insertion in the middle of the sequence to break the alpha-helical conformation. In contrast, variants with reduced numbers of leucine residues showed significantly reduced ability to promote interfacial adsorption and much worse activity under compression-expansion cycling. These results support the concept that hydrophobicity and potential leucine-promoted peptide-peptide interactions are more important than helicity for SP-B-like surface activity.

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2502-Pos Board B272

Interaction of Hydrophobic Surfactant Proteins with Oriented Phospholipid Bilayers

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The hydrophobic surfactant proteins, SP-B and SP-C (SPs), promote rapid adsorption of phospholipids to an air-water interface by a mechanism that remains unclear. To understand the structural changes that lead to faster adsorption, we measured small-angle X-ray diffraction from dipalmitoylphosphatidylcholine (DPPC) bilayers containing varying concentration of SPs on solid supports. Swelling at different hydration allowed interpretation of the diffracted intensities and construction of the electron density profile. With increasing concentrations of protein (0 - 10 wt/wt %), the d-spacing and bilayer thickness increased. This change may reflect stretching of the hydrophobic region of the bilayer caused by the presence of the protein. Larger amounts of protein also flattened the electron density profiles, reducing the difference between minimum and maximum densities. A disordering effect of the proteins should cause greater thermal fluctuations of the bilayer, which would explain both the flattened density profile and the observed loss of higher order Bragg peaks.

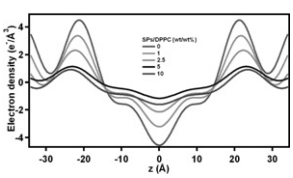

Figure 1: Electron density profiles of DPPC samples containing varying amount of SPs.

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Anionic Phospholipids change the Effect of the Hydrophobic Surfactant Proteins on Structures of Hexagonal Lipids

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Available evidence suggests that the hydrophobic surfactant proteins (SPs), SP-B and SP-C, accelerate adsorption of surfactant vesicles to an air/water interface by promoting formation of a negatively curved rate-limiting structure. In support of this model, the proteins induce several phosphatidylethanolamines to form inverse bicontinuous cubic phases, in which each leaflet has negative saddle-like curvature analogous to the hypothetical intermediate. The proteins could promote formation of cubic phases by changing spontaneous curvature (c_0) of lipids, which would be reflected in the dimensions of the inverse hexagonal (H_{II}) phase. With 1,2-dioleoyl phosphatidylethanolamine (DOPE), the SPs had no effect on the size of the H_{II} phase, suggesting a constant c_0. The study, however, lacked anionic phospholipids, which constitute ~10% (mol:mol) of phospholipids in lung surfactant, and could engage in selective interactions with the cationic SPs. In this work, we used small-angle X-ray scattering to examine how SPs affect structures formed by DOPE mixed with 10% (mol:mol) anionic 1,2-dioleoyl phosphatidylglycerol (DOPG). With DOPG, the H_{II} lattice-constant (a_0) decreased in a dose-dependent manner with increasing levels of protein. This change could be caused by specific interactions between the SPs and DOPG, or nonspecific interactions between cationic SPs and the anionic phosphate group of DOPG. To determine whether the observed change in a_0 was caused by nonspecific electrostatic effects, the measurements were repeated on samples prepared in buffered electrolyte. In the presence of counterions, the effect of SPs on a_0 was significantly diminished. These results suggest that the change in the a_0 for DOPE:DOPG in the absence of counterions is caused by nonspecific electrostatic interactions between the positively-charged proteins and anionic phospholipids, and are unlikely to play a major role in physiological media.

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2504-Pos Board B274

Effects of Hidrophobic Surfactant Proteins SP-B and SP-C on the Mechanical Properties and Structural Stability of Phospholipid Bilayers

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The respiratory surface is stabilized by pulmonary surfactant, a complex mixture of lipids and proteins, whose main function is reducing surface tension at the alveolar air-liquid interface in order to facilitate the work of breathing. It is composed by around 90% lipids and 8-10% specific proteins, including the hydrophobic polypeptides SP-B and SP-C. A combined action of both proteins is essential for a proper organization of functional membrane arrays in surfactant complexes.

SP-B and SP-C have dramatical effects on membrane structure and dynamics. In the present study we compare some structural, mechanical and dynamical properties of model POPC vesicles in the absence and presence of them, either in their physiological combined proportion or each protein by itself. Structural effects caused by hydrophobic surfactant proteins were noticed both by optical microscopy of giant proteolipid vesicles and by electron microscopy of 100 nm diameter extruded vesicles. Also, impermeable POPC membranes became permeable when supplemented with any of these proteins. Significant differences were noticed between the effect of SP-B and SP-C on giant vesicles: suspensions containing only SP-B were stable but those containing only SP-C were quite dynamic, undergoing frequent fluctuations, reorganizations and ruptures as observed under the microscope. In order to investigate the physical explanation of these phenomena, mechanical studies have been carried out with giant phospholipid and proteolipid vesicles.

The differences found between the effect of each protein separately and their physiological mixture support the concept that SP-B and SP-C mutually modulate their membrane-perturbing properties, which may be crucial for the structure, arrangement and dynamics of pulmonary surfactant membranes.

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Peptide-Lipid Reactivity in Membranes

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Acyl transfer from lipids to peptides is able to occur in the absence of enzyme catalysis. This innate reactivity is of fundamental interest, with the potential to influence a number of membrane processes. The kinetics and selectivity of the